DNA Topical Conference
Room 311 - Session DN+BI-MoA

DNA Detection and Sensing

Moderator: R.M. Georgiadis, Boston University

2:00pm **DN+BI-MoA1 Diamond-based Electrical Biosensors for DNA Detection**, ***B. Sun***, *W. Yang, H. Kim, K.-Y. Tse,* University of Wisconsin-Madison*; J.N. Russell, Jr., J.E. Butler,* Naval Research Laboratory*; R.J. Hamers,* University of Wisconsin-Madison

The high stability of diamond makes an attractive material to use for interfacing microelectronics to biological molecules such as DNA. We have investigated the fabrication of diamond-based field-effect transistors in which the surface of the diamond is functionalized with biomolecules of interest, and binding to target molecules in solution produces a change in electrical properties via a field effect. Because the sensitivity depends on the size of the FET, our efforts have been placed on developing small devices, a few microns in size. Measurements in a field-effect transistor geometry can be compared to those made via impedance spectroscopy, as both are sensitive to the impedance of the diamond space-charge region. This talk will discuss the factors controlling the sensitivity and electrical stability of diamond-based electrical biosensing devices, as applied to DNA and other biomolecules.

2:20pm **DN+BI-MoA2 Polymer Replicated Interdigitated Electrode Arrays and Their Application in Multiparameter Molecular Diagnostics**, ***W. Laureyn***, *J. Suls, K. Bonroy,* IMEC, Belgium*; G. Van Reybroeck, P. Jacobs, R. Rossau,* Innogenetics NV, Belgium*; P. Detemple,* IMM GmbH, Germany*; C. Van Hoof,* IMEC, Belgium

The development of DNA-sensor devices attracts substantial research efforts directed to gene analysis, detection of genetic disorders, tissue matching, forensic applications, etc. The electronic transduction of the formation of nucleic acid/DNA complexes using electrodes or semiconductors could provide quantitative information on the DNA-analyte in the sample. InterDigitated Electrode (IDE) arrays show great promise for the label-free detection of nucleic acid hybridization. However, the search for a technology that allows the manufacturing of thin-film IDE arrays on polymers in an easy and affordable fashion, which is compatible with state-of-the-art microfluidics integration technology, has received little attention to date. This paper reports on an innovative method for the affordable manufacturing of polymer based arrays of IDEs with µm dimensions. The reported fabrication process is based on a single and directional metal deposition on an appropriate three-dimensional structure, which is realized in a polymer material using micro-injection molding. The molds are manufactured by electroplating as a reverse copy of a silicon master structure. Using a well-designed combination of so-called microchannels and bumps, a self-contained shadowing effect can be achieved resulting in separated IDE structures after directional metal deposition. In this paper, we report on the full experimental proof-of-principle of the production of such devices and on their further integration in a functional micro-fluidic device. As a demonstrator application, we are currently investigating the label-free detection of post-amplification nucleic acid targets. Using planar 1µm IDEs on silicon, fabricated using deep-UV lithography, we were able to discriminate the hybridization of a 1 nM, 261-nt-long PCR-sample (exon 2 of HLA-DQB) down to a single-base mismatch level. A proof-of-principle on DNA-detection with the molded polymer structures is expected mid 2005.

2:40pm **DN+BI-MoA3 Surface Enzyme Reactions for Enhancing SPR Imaging Measurements of DNA Microarrays**, ***R.M. Corn***, University of California, Irvine **INVITED**

The identification and application of bioaffinity interactions in a large scale array format has become an indispensable tool for modern biological research. Bioaffinity interactions such as DNA-DNA and DNA-protein interactions are now employed in an array format to quickly ascertain the presence of a particular DNA or RNA sequence in a sample, to detect and identify microbial and viral species, and to verify efficacy and function in medical diagnostics. In addition to the detection of DNA, microarrays are can be for the identification of new DNA-protein bioaffinity interactions such as and protein-protein binding. The surface-sensitive optical technique of surface plasmon resonance (SPR) imaging is a powerful "label free" measurement that can be used in an array format for the detection of bioaffinity interactions. SPR imaging detects the presence of a biopolymer on a chemically modified gold surface by the change in the local index of refraction that occurs upon adsorption. This talk will highlight the use of surface DNA enzyme reactions in conjunction with SPR imaging measurements to either provide enhanced biochemical selectivity or to amplify the optical response of the bioaffinity adsorption events. For example, we have recently used the enzyme RNase H to detect DNA adsorption onto RNA microarrays from femtomolar solutions. Further experiments on the reaction of Exo III with double-stranded DNA arrays and the use of Exo I with single-stranded DNA arrays will be employed to detail the kinetics of the surface enzyme reactions, which can be described with a combination of Langmuir and Michaelis-Menten concepts.

3:20pm **DN+BI-MoA5 Electrically Switchable DNA Layers as a Novel Detection Scheme for Bio-Sensing**, ***U. Rant***, *K. Arinaga, E. Pringsheim, M. Grubb,* University of Technology Munich, Germany*; S. Fujita, N. Yokoyama,* Fujitsu Laboratories Ltd., Japan*; M. Tornow, G. Abstreiter,* University of Technology Munich, Germany

Recently, we reported on the electrical manipulation of oligonucleotide layers tethered to gold surfaces.@footnote 1@ By applying alternating AC potentials to the supporting substrate, the orientation of the DNA strands can be dynamically switched with frequencies ranging up to the kHz regime, while the layer conformation is probed in real-time by optical means. Here we present the underlying principles governing the switchability of DNA layers and, in particular, how this novel functionality can be employed for multi-purpose, label-free sensing applications. In principle, the method can be utilized to detect any kind of (bio-) molecules that, upon specifically binding to the grafted probe layer will alter its switching behavior. As a unique feature, the technique allows to monitor the molecular dynamics of the electrically switched layers, which provides novel means to identify and characterize target-probe complexes on surfaces. We demonstrate the versatility and high sensitivity of the technique by the recognition of specific DNA sequences as well as the detection of a model protein system. @FootnoteText@ @footnote 1@ U Rant, K Arinaga, S Fujita, N Yokoyama, G Abstreiter, M Tornow; Nano Letters 4 (2004), 2441-2445

3:40pm **DN+BI-MoA6 DNA Conductance Sensor Platforms Using Nano-scale Break Junctions**, ***A.K. Mahapatro***, *K.J. Jeong, S. Bhattacharya, G.U. Lee, D.B. Janes,* Purdue University

For DNA sensors, a direct electrical readout of DNA selective binding events would enable integration of sensor elements with readout circuits. A possible readout approach involves measurement of the electrical conductivity of DNA strands bridging two narrowly spaced metallic contacts. In this work we describe few-molecule conductance measurements with electromigration-induced break-junctions (EIBJ). The double-stranded(ds) DNA oligionucleotide sequences are GGCGCGCGGGCGGGC-(CH@sub2@)@sub3@-SH-3', GGCGCAAAAACGGGC-(CH@sub2@)@sub3@-SH-3', and HS-(CH@sub2@)@sub6@-CGGAGAGTTGAGCAT-3', and their complements. Lithographically defined Au wires are formed by e-beam evaporation over oxidized silicon substrates silanated with (3-Mercaptopropyl)trimethoxysilane (MPTMS), then subjected to electromigration at room temperature to create nano-gaps. Although the Au wires are initially 2µm wide, gaps with length ~1nm and width ~5nm are observed after breaking, as observed through a field effect scanning electron microscope. ds-DNA was immobilized on the electrodes by assembling the DNA double-strands in an aqueous solution, reacting these solutions with the electrodes in solution, locking the double-helix configuration with a polycation, thorough rinsing with ultrapure water to remove any residual salt, and drying before measurement. The GC-rich, 3' thiol labeled DNA showed approximately 1Gohm resistance, but little conductivity was measured in the AT-rich or 5'thiol labeled DNA. This is consistent with single molecule conduction measurements where enhanced conductivity has been observed in GC-rich DNA. For the GC-rich DNA, higher conductivity is observed for devices immobilized in a higher concentration of salt (NaCl) in the standard phosphate buffer solution, which is attributed to more DNA-molecules immobilized between the electrodes. This study demonstrates that the EIBJ technique can be used to understand the electrical properties in nanometer scale materials such as DNA.

4:00pm **DN+BI-MoA7 Detection of DNA Hybridization on Porous Silicon Surface by Infrared Microspectroscopy**, ***R. Yamaguchi***, *K. Ishibashi, K. Miyamoto, Y. Kimura, M. Niwano,* Tohoku University, Japan

We propose a label-free method of detecting DNA hybridization by using porous silicon (por-Si) in conjugation with infrared (IR) microspectroscopy. In our method, DNA hybridization is detected through an analysis of infrared spectral profiles, and therefore fluorescence tags are not necessary for the hybridization detection. By using a por-Si film as the chip

substrate, we can immobilize a great number of DNA molecules in a small surface area on the chip surface, since por-Si has a quite large effective surface area as compared to a flat Si surface. This may facilitate a high-sensitive detection of DNA hybridization on a small spot. In this study, we have investigated the feasibility of our method by measuring infrared absorption spectra of DNA molecules on por-Si film surfaces. We prepared a por-Si film with straight pores by anodizing a heavy-doped n-type silicon (100) wafer in a mixture of dilute hydrofluoric acid and ethanol. The diameter of straight pores was approximately 25 nm. Single-stranded DNA (target DNA) with 20 bases was immobilized on the por-Si film surface, and then the film surface was exposed to two kinds of probe DNA; one is complementary to the target DNA, and the other is not complementary to the target DNA. We utilized an IR microspectrometer to measure IR absorption spectra of the film before and after exposure to probe DNAs. The working area of the microspectrometer was set at 50Ã-50 Âµm2. For complementary DNA, we observed absorption peaks due to the probe DNA even after the film surface was rinsed with sodium chloride solution. For non-complementary case, on the other hand, no spectral changes were observed. These observations imply that DNA hybridization can be detected using IR microspectroscopy in conjugation with a por-Si based chip.

4:20pm **DN+BI-MoA8 Probing DNA-DNA Interactions between Cytosine (dC) Homo-Oligonucleotides Immobilized on Gold**, ***A.M. Opdahl,*** National Institute of Standards and Technology; *D.Y. Petrovykh,* University of Maryland and Naval Research Laboratory; *H. Kimura-Suda,* National Institute of Standards and Technology; *L.J. Whitman,* Naval Research Laboratory; *M.J. Tarlov,* National Institute of Standards and Technology

We present experimental evidence for strong interactions between cytosine (dC) homo-oligonucleotides immobilized on gold surfaces. It is known that in neutral and acidic pH solutions (dC)-rich oligos can form multistrand structures [e.g. parallel strand, i-motif] via hemiprotonated (C+)(C) base pairing. For surface-immobilized DNA, we find evidence for the existence of these structures by probing the susceptibility of oligo(dC) films to displacement by 1-mercapto-6-hexanol (MCH) as a function of the buffer solution pH and ion composition. The premise of the method is that MCH is less effective at displacing any individual oligos within a film when strong DNA-DNA interactions are present. The structures and coverages of alkanethiol modified (-SH) and unmodified oligo(dC) films were characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Specifically, we find that (dC)@sub 25@-SH films are not displaced by MCH in neutral and acidic pH conditions that favor base-base (C+)(C) interactions, but are readily removed by MCH in mildly basic conditions. Both thiol modified and unmodified (dC) films exhibit higher resistance to MCH displacement in the presence of divalent buffer cations. This additional stability is attributed in part to electrostatic crosslinking of the negatively charged phosphate backbones. These results will be discussed along with the possibility of using (C+)(C) base pairing for stabilizing thiol-tethered DNA strands on gold.

4:40pm **DN+BI-MoA9 Quantification of Immobilized and Hybridized Oligonucleotide Surface Density on Commercial Amine-Reactive Microarray Slides using Radiometric Assay, Fluorescence Imaging and X-Ray Photoelectron Spectroscopy**, ***P. Gong,*** *G.M. Harbers, D.W. Grainger,* Colorado State University

In an effort to establish a quantitative understanding of the correlation between immobilized probe DNA density on microarray surfaces and target hybridization efficiency in biological samples, we have characterized amine-derivatized, single-stranded DNA probes attached to amine-reactive commercial microarray slides and its complementary DNA target hybridization using fluorescence imaging, X-ray photoelectron spectroscopy (XPS) and @super 32@P-radiometric assays. Importantly, we have reproduced immobilization efficiencies of DNA probes under microarray formats using high ionic strength and increased DNA concentrations in macroscopic spotted dimensions to permit XPS surface analysis with good reliability and reproducibility. Target hybridization efficiency with complementary DNA was studied on these capture surfaces and shown to exhibit an optimum at intermediate probe densities. The macroscopic model provides a new platform for study of DNA surface chemistry using highly sensitive, quantitative surface analytical techniques. (e.g., XPS, ToF-SIMS) Sensitive @super 32@P-DNA radiometric measurements are now calibrated with more routine XPS DNA signals, facilitating future routine DNA density determinations without the use of hazardous radioactive assay. The objective is to provide new insight into the surface chemistry influences on DNA probe environments that influences the efficiency of target capture form solution in order to improve microarray assay performance.

5:00pm **DN+BI-MoA10 Ion Current Detection of Mono-nucleotide Passing into a Nano-hole Fabricated on Si Wafer**, ***A. Oki,*** *Y. Horiike,* National Institute for Materials Science, Japan

D. Branton et al. demonstrated the electrical sequencing based on measurement of currents generated by passage of single-strand DNA into a 2 nm hole drilled in a cell membrane. But about 5 nm thick membranes do not allow discriminating single molecule of DNA with stacking spacing of 0.34 nm. For the goal, we have studied detection of currents generated from one base flowing into the nano-hole after cutting DNA to each base using a reaction of @lambda@exonuclease. The nano-hole was fabricated as follows: First, the KOH etching fabricated an anisotropic feature on the backside of the wafer through a 44 µm square Si@sub 3@N@sub 4@ mask, thus self-stopping at 31 µm depth. Then, the KOH etching opened an anisotropic feature on the upper side masked by a 5 mm square window, thereby forming a 360 nm diameter hole on the bottom of the upper side. 50 nm thick SiO@sub 2@ film grown on Si and subsequent CVD of 150 nm thick Si@sub 3@N@sub 4@ film filled periphery of the hole. Finally, 4.5 KeV Ar@super +@ ion irradiation removed the Si@sub 3@N@sub 4@ film on the hole position, thus fabricating the nano-size hole by monitoring instant increase of the ion current using a micro-channel plate set under the wafer. The hole size was not observed by SEM. To measure ion currents generated by mono-nucleotide passing the nano-hole, each 500 µM natural mono-nucleotide of dGMP, dCMP, dAMP, TMP was solved in a TE buffer solution, where a pair of a KCl saturated calomel was used as electrodes. Each ion current increased with increasing voltage. At 1.5V, ion currents of dCMP, TMP, dGMP, and dAMP were 45.9, 21.5, 15.5 and 13.8 nA, respectively. If the ion current varied inversely with molecular weight of mono-nucleotide, increases in order of ion currents are understood for dCMP and TMP. However present experimental accuracy must be checked for inverse characteristic of dGMP for dAMP because of small difference between both ion currents.

Author Index

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